

**Amendments to the Specification:**

Please replace the paragraph at page 13, lines 4 to 23 with the following amended paragraph:

C1 To determine if the protease has been secreted in an active form, a sample of the bacterial culture is provided with chosen potential substrates and cleavage of these substrates is determined. For example, a bacterial pellet from Step 3 is gently resuspended in 500  $\mu$ l of DMEM in the presence of common extended substrates (myosin II, bovine serum albumin, collagen, fibronectin, and hemoglobin). Myosin II, but not the proteins from milk such as casein, has been shown to be a suitable substrate to measure the proteolytic activity of gram positive bacteria isolated from dried cured ham (Rodriguez et al., [J Appl] J. Appl. Microbiol. 1998. Nov; 85(5):905-12). The suspension of bacteria and substrates is incubated at 37°C with gentle shaking. At preset times (0.1, 0.3, 1.0, 3.0, 5.0, 24, and 48 hours) the samples are centrifuged to spin down the bacteria, and a small aliquot is removed for a SDS-PAGE gel sample. After completion of the time course the samples are run on a 10-15% gradient SDS-PAGE minigel. Then, the proteins are transferred to Immobilon Pseq (Transfer buffer, 10% CAPS, 10% methanol pH 11.0, 15 V for 30 minutes) using a [Bio-Rad] BIO-RAD semi-dry transblotting apparatus. Following transfer of the proteins, the blot is stained with Coomassie blue R-250 (0.25% Coomassie Brilliant Blue R-250, 50% methanol, 10% acetic acid) and destained (high destain for 5 minutes, 50% methanol, 10% acetic acid; low destain until complete, 10% methanol, 10% acetic acid) followed by sequencing from the N-terminal. Alternatively, the samples will be run on a mass spectrometer in order to map the sites of proteolytic cleavage using a Voyager Elite Mass spectrometer (Perspective Biosystems).

Please replace the paragraph at page 15, line 24 to page 16, line 13 with the following amended paragraph:

C2 When the enzyme is a metalloprotease (mpl), the active form of the mpl can be amplified by polymerase chain reaction using forward and reverse primers containing useful restriction sites for cloning into an expression vector with a strong inducible promoter (such as the T7 system of pET-28a, [Novagen] NOVAGEN). Useful primers for the amplification are catgccatgggtagaacgggctgataccca (SEQ ID NO. 4) and ggcgcggaattctcagttaacccaactgctt (SEQ ID

C2  
Complete

NO. 5). Each primer has at least a 6 base pair overhang. The DNA is amplified from either lysed colonies of *L. monocytogenes* or from purified preparations of genomic DNA from *L. monocytogenes*. The forward primer is provided with a *Nco* I site that includes the start methionine (CATATG). The reverse primer includes a stop codon (TGA) and an *Eco* RI restriction site (GAATTC). A typical amplification protocol comprises amplifying the genetic sequence with Taq polymerase under the following conditions: 92°C initial melt 4 minutes (25 cycles, 50°C anneal 30 seconds, 72°C extension 1.5 minutes, 92°C melt 30 seconds), 72° C final extension 10 minutes and 4°C store. The annealing temperature may be increased or decreased somewhat to account for the predicted melting temperature ( $T_m$ ) of the primers. Following cloning of the genetic sequence for the active metalloprotease into pET28a, the gene is sequenced by double stranded primer walk sequencing on an ABI310 genetic analyzer. The recombinant active form of the metalloprotease genetic sequence is purified by nickel-nitrilotriacetic acid chromatography by taking advantage of the polyhistidine tag in the pET-28a vector.

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Please replace the paragraph at page 16, line 19 to page 17, line 2 with the following amended paragraph:

C3

For example, a chromagenically labeled peptide could be bound to a polyvinylidene fluoride (PVDF) membrane with a hydrophobic area. When the peptide is clipped, the dye would concentrate in the hydrophobic area, becoming visible. Alternatively, the peptide could be labeled fluorescently, and visualized with a UV lamp. Another possibility is a peptide with a chromagenic leaving group that upon removal changes color. The peptide may be labeled with a blue dye, coupled to a red surface. To the observer, the surface would look black unless the specific bacteria were present to provide protease that would cleave the peptide, turning the surface red as the peptide was proteolysed and diffused away. Although conventional leaving groups such as 4-nitroaniline or 7-amino-4-methyl-coumarin can be quantified spectrophotometrically and [fluorometrically] fluorometrically, it may be best to use a leaving group such as Texas Red that has an intense blue color. By placing two or three Texas Red groups near the end of the peptide, it would be possible to detect visually the clearance of the substrate by *L. monocytogenes* protease activity.

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Please replace the paragraph at page 17, line 24 to page 18, line 9 with the following amended paragraph:

cy Optionally unoccupied reactive sites on the microtiter plate or on the food packaging are blocked by coupling bovine serum albumin, or the active domain of p26 thereto. p26 is an alpha-crystallin type protein that is used in this case to reduce non specific protein aggregation. The ability of the p26 protein to refold heat denatured citrate synthetase before and after coupling to the surface of the food packaging is used as a control for determining p26 activity. Alpha-crystallin type proteins were recombinantly produced using standard recombinant DNA technologies (Maniatis et al., Molecular cloning: a laboratory manual. 1982). Briefly, the plasmid containing the beta sheet-charged core domain of p26 is electroporated into electrocompetent B121 (DE3) cells ([Bio-Rad] BIO-RAD E. coli pulser). The cells are grown up to an OD of 0.8, then induced with 1 mM IPTG for 4 hours. The cells are spun down, and sonicated in low buffer (10 mM Tris, pH 0.8, 500 mM NaCl, 50 mM Imidazole) to lyse (Virsonic, Virtis, Gardiner, NY). The lysate is spun down at 13,000 x g for 10 minutes, and the supernatant 0.45 and 0.2  $\mu$ m filtered. This filtrate is loaded onto a Ni-NTA superose column ([Qiagen] QIAGEN, Valencia, CA, cat # 30410). High buffer (10mM Tris pH 8.0, 500 mM NaCl, 250 mM Imidazole) is used to elute the protein.